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13. ABSTRACT *(Maximum 200 words)*

In order to evaluate laser safety standards, Dr Toth examined fluorescein angiograms (in some cases OCT) and fixed tissue sections and aided in defining exposure limits for pulsewidths less than one nanosecond. In our initial short pulse study, we were concerned with the restriction to a single wavelength. To verify that the tissue effects were due to the pulse structure, we obtained data outside a single wavelength. We obtained from information from -530-nm and -1060nm wavelengths to compare to data obtained from previous studies of visible laser picosecond and femtosecond pulses. The type of lesions created with 530 and 1060- 1064nm wavelengths were similar to the lesions created in the earlier 580-nm wavelength studies. This supported the theory that laser induced breakdown is one of the primary damage mechanisms for ultrashort laser pulses. Data from our studies was used by the American National Standard Institute in publications ANSI Z136.1, "Safe Use of Lasers" (updated 2000) and ANSI Z136.3, "Safe Use of Lasers in Health Care Facilities"(1996).

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I. FINAL REPORT

EFFECTS ON OCULAR TISSUE OF MULTIPLE LASER PULSES AND OF SINGLE ULTRASHORT PULSES OF VARYING WAVELENGTH

Grant Number: F4692-98-1-0412

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Reporting Period:

01 September 1998 - 31 August 2001

II. OBJECTIVES:

Unchanged from Statement of Work.

III. STATUS OF EFFORTS:

A. Retinal Lesions from Single Ultrashort Laser Pulses of ~1060-nm and ~530-nm wavelength:

In order to evaluate the safety from ocular exposure to ultrashort pulses, Dr. Toth coordinated the angiographic and histopathologic studies, sectioned and analyzed tissue to define exposure limits for pulsewidths less than one nanosecond. [1] Working with the Armstrong Laboratory group, Dr. Toth proposed a study of ultrashort laser retinal lesions created by different wavelengths. Dr. Toth collaborated with Armstrong Laboratory (Dr. Cain et al.) in analyzing near-infrared ultrashort laser pulse injury MVL data. These studies have been completed and the results published. [2]

Large Spot Size Lesions: We looked at the pattern of retinal injury from focal sites of injury scattered within large spot size ultrashort laser single pulses. In this study we compared the retinal damage thresholds of large spot laser lesions in the near infrared range to single small spot laser lesions from near infrared and visible wavelengths reported previously. [3] We found multiple foci of damage within the area of larger ultrashort single pulse lesions. These studies have been completed and the results published. This is the first report of this phenomenon in living tissue. [4]

Sub-50 Femtosecond Lesions: Previous studies reported the retinal effects caused by single and multiple ultrashort laser pulses from visible and near infrared wavelengths. [2,5-6] In this study we analyzed retinal damage from sub-50fs laser lesions and compared them to longer pulses. We have received and processed the following primate tissue: sub-50fs, chirped, 810-nm single pulse lesions and sub-50fs, flat-phase, 810-nm, single pulse lesions. Initial studies of sub-50fs lesions were presented at the Association for Research in Vision and Ophthalmology 2001. [Abst 1] On analysis of that data, we recognized a need for additional lesions created by sub-50fs laser to allow for meaningful analysis. We requested and recently received tissue treated with high and low energies of sub-50fs laser. We are in the process of evaluating these final specimens. I expect a report of these lesions to be completed by our laboratory by 1 December, 2001.

Long-Term (Chronic) Laser Lesions: We looked at the long-term healing response of retina after exposure to laser. We completed the histopathologic evaluation of a limited number of 4 month, 1 year, 2 year, and 4-year old lesions and compared these findings to those from acute lesions. Histopathologic evaluation of the chronic lesions was difficult in that only 68 of the 410 lesions delivered were visible on light micrographs. We believe that the retinal healing response resolved the remaining lesions prior to enucleation. Analysis of this data has been completed.

B. Multiple Pulse Laser Retinal Effects:

In an effort to document the extent of retinal effects caused by multiple pulse laser pulses, we compared the effects of multiple pulse acute and chronic lesions to the effects of single pulse acute and chronic lesions. We have completed the histology and analysis of the following primate tissue: 80-ps, 1064-nm single-pulse chronic lesions; 130-fs, 800-nm 100-pulse lesions; 20-ps, 1064-nm single-pulse chronic lesions; and 130-fs, 800-nm 1000-pulse lesions. This data was analyzed and published in collaboration with the Armstrong Laboratory group.[6]

Mode-locked vs. Continuous Wave Lesions: In an attempt to provide a direct comparison between the effects of mode-locked ultrashort pulse laser lesions to the effects of continuous-wave laser lesions, we evaluated tissue from an experiment where the ML and CW lasers possessed identical output characteristics. We have completed the histology and analysis of the following primate tissue: 130-fs, 0.25sec., acute and chronic mode-locked lesions; 800-nm, 0.25sec., acute and chronic continuous wave lesions; 130-fs, 800-nm, single pulse lesions; 130-fs, 800-nm, 10-pulse lesions; 130-fs, 800-nm, 10,000-pulse lesions; 1-ps, 1060-nm, single pulse, long-term lesions; 150-fs, 1060-nm, single pulse, long-term lesions; and 7-ns, 1064-nm single pulse, long-term lesions. Our publication of this data is in submission in Lasers in Surgery and Medicine.(attachment 1)

C. Retinal Models:

Porcine Tissue as an Alternate Animal Model: We researched the utility of the porcine eye as an animal model that provides information pertinent to the human eye at risk from laser exposure. The summary report of preliminary evaluation of the porcine eye for possible laser safety evaluation was provided to the Air Force and Army Brooks laser research groups on 30 October, 1998. These groups have utilized this data in considering future animal studies. Dr. Michael Jumper (Wilford Hall USAF Medical Center) utilized this information and Dr. Toth's consultation in preparing a laser study at the animal research facility at Wilford Hall. His work is utilizing a porcine model for evaluation of retinal laser effects. At Duke University Medical Center, Dr. Toth provided information to Dr. Sharon Fekrat, who also utilized a porcine eye model for testing of novel laser methods to create retino-choroidal anastamosis after vascular occlusion.

Retinal Laser Lesions Examined by OCT Before and After Fixation: To determine the effects of fixation on retinal pathology, we studied excised porcine and primate tissue containing acute and chronic laser lesions. The porcine tissue was fixed in either gluteraldehyde or formalin. The primate tissue was fixed in gluteraldehyde. All evaluations were performed with Optical Coherence Tomography and histopathologic analysis. We found that OCT was useful in localizing laser lesions prior to sectioning. Fixation induced several specific changes in the OCT imaging of the tissue. This data was published in SPIE proceedings.[7]

We have worked diligently at publishing the results of our research in the ophthalmic and engineering literature.

IV. ACCOMPLISHMENTS / NEW FINDINGS:

A. Retinal Lesions from Single Ultrashort Laser Pulses of ~1060-nm and ~530-nm wavelength:

In order to evaluate laser safety standards, Dr. Toth examined fluorescein angiograms (in some cases OCT) and fixed tissue sections and aided in defining exposure limits for pulsewidths less than one nanosecond. In our initial short pulse study, we were concerned with the restriction to a single wavelength. To verify that the tissue effects were due to the pulse structure, we obtained data outside a single wavelength. We obtained information from ~530-nm and ~1060-1064nm wavelengths to compare to data obtained from previous studies of visible laser picosecond and femtosecond pulses. The type of lesions created with 530 and 1060-1064nm wavelengths were similar to the lesions created in the earlier 580-nm wavelength studies. This supported the theory that laser induced breakdown is one of the primary damage mechanisms for ultrashort laser pulses. Data from our studies was used by the American National Standard Institute in publications ANSI Z136.1, "Safe Use of Lasers"(updated 2000) and ANSI Z136.3, "Safe Use of Lasers in Health Care Facilities"(1996).

Large Spot Size Lesions: This grant term we identified a novel pattern of retinal injury (focal sites of injury scattered within a single large lesion) from large spot size ultrashort laser single pulses. There was a pattern of multiple focal sites of retinal damage across the area of laser beam delivery. These multiple punctate sites of visible retinal damage were seen on clinical examination of the fundus and in light micrographs of the fixed tissue. There was also a different choroidal effect in large spot size lesions when compared to smaller size lesions. This is not consistent with a strictly thermal model of laser injury. Self-focusing filamentation of the laser beam as it traveled to the retina may be responsible for the multiple lesions. This is the first report of this phenomenon in living tissue.[4]

Sub-50 Femtosecond Lesions: We compared the effects of 44-fs, pre-chirped, 810-nm near-infrared single laser pulses to 90-fs, ultrashort laser lesions and 150-fs &130-fs near-infrared laser lesions. Although similar in appearance to many of the low energy focal outer retinal lesions from the 90-150-fs range, the 44-fs, pre-chirped lesions demonstrated more localized, smaller areas of damage and no choroidal effect. There was no thermal spread across a range of energies up to 4 fold what is required to make a visible lesion. As we have moved to shorter pulsewidths, due to the minimal energy per pulse, we have seen focal damage limited to the outer retinal layers. This data is preliminary due to the small number of lesions and limited range of energies delivered. The study will be completed upon analysis of the final tissue now in our laboratory.

Long-Term (Chronic) Laser Lesions: We evaluated the retinal change in long-term (chronic) laser lesions. We were unable to find the four-year-old lesions in our light

micrographic sections. We did, however, find a few of the lesions delivered 4 months, 1 year, and 2 years prior to enucleation. Of the 410 lesions delivered, we were able to find 6 of 26 (23.1%) 4 month old lesions, 35 of 239 (14.23%) 1-year old lesions, 28 of 125 (22.4%) 2-year old lesions, and 0 of 20 4-year old lesions. Most of the visible chronic lesions were similar in appearance regardless of lesion age. As expected, we found significant change in the appearance of chronic lesions when compared to acute lesions. The chronic lesions showed compression of the photoreceptor layer with little retinal pigment epithelial damage. The remaining damage that was visible by light microscopy did not extend into the outer plexiform layer. This data is useful in demonstrating the extent of retinal healing after laser exposure.

B. Multiple Pulse Laser Retinal Effects:

There has been an increase in the number of portable lasers. Most pulsed lasers are multiple pulse rather than single pulse systems. In this study we correlated the change in ocular pathology with the change in number of pulses, total energy and change in time between pulses for multiple short laser pulses delivered to the retina. We found that multiple pulse lesions were similar in appearance to single pulse lesions.[6] However, the extent of retinal injury was more dependent on the energy delivered to the retina than the number of pulses. The multiple pulse lesions showed thin columns of damage with RPE involvement. Higher energy pulses caused full thickness retinal injury. Hemorrhage was visible at higher energies. There was no visible choroidal damage.

Mode-locked vs. Continuous Wave Lesions: We compared the extent of retinal and choroidal damage in mode-locked and continuous wave lesions. The acute pattern of thermal injury is grossly similar after cw and mode-locked laser treatment of the retina. We did find a slight difference in lesion size and in pattern of nuclear injury between matched lesions in the two groups. This raises the question of some unexpected aberrations in the beam delivered to the retina. This could either be caused by laser or by mechanisms of injury in addition to the thermal damage manifested in these lesions. Additional photochemical injury or photoreceptor nuclear damage from the mode-locked laser cannot be ruled out on the basis of this study. To resolve this question of effect, future biochemical studies of photoreceptor nuclei after application of mode-locked laser energy vs. cw laser energy would be needed. Our publication of this data is in submission in Lasers in Surgery and Medicine.(attachment 1)

C. Retinal Models:

Porcine Tissue as an Alternate Animal Model: Although the non-human primate eye is an optimal model because of the fovea, optics and similarity to the human eye, the porcine eye was found to be adequate for preliminary studies. The optics, lateral magnification and axial magnification of the porcine eye measure more closely to the human than the primate. However, the porcine eye does not have a fovea, instead there is an area centralis or macular streak. The porcine eye has a fully vascular retina like the human and primate. This is critical in human laser studies because

damage to the macula and laser-induced hemorrhages are two main damage events with the greatest visual impact in humans. We propose that porcine eyes could replace the major use of the primate model in a large number of laser ocular safety studies. The summary report of preliminary evaluation of the porcine eye for possible laser safety evaluation was provided to the Air Force and Army Brooks laser research groups on 30 October, 1998. These groups have utilized this data in considering future animal studies. Dr. Michael Jumper (Wilford Hall USAF Medical Center) utilized this information and Dr. Toth's consultation in preparing a laser study at the animal research facility at Wilford Hall. His work is utilizing a porcine model for evaluation of retinal laser effects. At Duke University Medical Center, Dr. Toth also provided this information to Dr. Sharon Fekrat, who also utilized a porcine eye model for testing of novel laser methods to create retino-choroidal anastamosis after vascular occlusion. (Abstract 2)

Retinal Laser Lesions Examined by OCT Before and After Fixation: We tested Chauhan and Marshall's proposal that fixed retinal tissue imaged similar to living tissue on optical coherence tomography. Unlike Chauhan and Marshall, we found that OCT imaging of excised retinal tissue is affected by formalin and glutaraldehyde fixation. We also found that OCT imaging can be performed on excised retinal tissue to identify the location of laser lesions. This may be helpful in locating lesions for histopathology. This data was published in SPIE proceedings.[7]

V. PERSONNEL SUPPORTED:

Personnel:	Job Title:
Cynthia A. Toth, M.D.	Principal Investigator
Ewa Worniallo	Histology Laboratory Analyst
Katrina P. Winter	Research Analyst
Michelle N. McCall	Research Analyst

Collaborators:

Dr. Clarence P. Cain TASC, San Antonio, TX

Dr. Cain developed and maintained the ultrashort lasers necessary for this research (in collaboration with Drs. Rockwell and Roach, and with Mr. Noojin). Dr. Cain and Dr. Toth collaborated in the design of the laser lesion placement for the MVL studies. Dr.Toth evaluated the fluorescein angiography of laser lesions in the primate retina. This changed the concept that fluorescein angiograms are more sensitive for the identification of retinal injury and support novel theories of ultrashort laser effects.

Maj. Cheryl D. DiCarlo USUHS, Bethesda, MD

Dr. DiCarlo provided essential support in the design and implementation of safe appropriate animal management for these studies. Dr. Toth provided specialized information regarding inducing ocular akinesia and ocular anatomy, useful for Dr. DiCarlo's studies.

Lt. Daniel X. Hammer Armstrong Laboratory, Brooks AFB, TX

Dr. Hammer assisted with the studies of minimal visible lesions and was a co-inventor of a laser probe.

Gary D. Noojin TASC, San Antonio, TX

Mr. Noojin provided essential laser design skills necessary for the creation of the ultrashort laser pulses. He also participated in the invention of the laser probe.

Maj. William P. Roach AFOSR, Bolling AFB, DC,

Dr. Roach designed the ultrashort laser research program and collaborated with Dr. Toth on the experimental design for the analysis of the impact of ultrashort laser pulses in the retina. His expertise in laser physics was necessary for the design and implementation of this work. Dr. Toth provided essential data regarding ocular structures, pathophysiology and histopathologic effects that were essential to Dr. Roach's research. This has made the Armstrong-Duke project a true partnership in the study of laser effects. He also participated in the invention of the laser probe.

Dr. Benjamin A. Rockwell Armstrong Laboratory, Brooks AFB, TX

Dr. Rockwell has organized the Armstrong laser program for the study of ultrashort effects on the eye. He provided essential laser physics expertise necessary for the collaborative studies of retinal effects of ultrashort laser pulses. Dr. Toth provides information regarding ocular media, pathophysiology, and interpretation of studies in light of visual needs. Dr. Toth assists Dr. Rockwell's group with laser study planning and lesion placement.

David J. Stolarski TASC, San Antonio, TX

Mr. Stolarski continued the laser delivery work of Dr. Cain and managed the delivery of laser to retinal sites. He worked closely with Ms. McCall and Winter of Dr. Toth's laboratory to ensure the smooth transport of ocular tissues for evaluation.

Other Academic Collaborators:

Reginald Birngruber, Ph.D. Medizinisches Laserzentrum Lubeck

Dr. Birngruber collaborated on the optical coherence tomographic imaging of retinal laser lesions.

Stephen A. Boppart, MSEE Massachusetts Institute of Technology

Dr. Boppart collaborated on the optical coherence tomographic imaging of retinal laser lesions.

Vincent P. Deramo, M.D. Duke University Eye Center

Dr. Deramo collaborated in the placement of lesions and the evaluation of fixation effects on OCT of laser lesions in the porcine eye.

Thomas Eurell, DVM, PhD Coll. Vet. Med., University of Illinois

Dr. Toth consulted and provided background information for proposed research of cellular and molecular mechanisms governing the ocular tissue response to ultrashort (ps & fs) pulse laser exposure.

Sharon Fekrat, M.D. Duke University Eye Center

Dr. Toth provided information into the use of a porcine eye model to test novel high-energy laser methods to create retino-choroidal anastamatosis after vein occlusion. This model was implemented in preliminary animal studies performed at NC State University, reported at ARVO in 2000. (Abstract 2)

James G. Fujimoto, Ph.D. Massachusetts Institute of Technology

Dr. Fujimoto collaborated on the optical coherence tomographic imaging of retinal laser lesions.

Robert Guenther, PhD Duke Free Electron Laser Center

Information gained from the OCT studies of the anterior segment in the ultrashort laser program at Brooks[8] was utilized to design a study using OCT to examine the fibrin and wound-healing response after Free electron laser incision of the cornea. Dr. Guenther appreciated the utility of the OCT imaging and has Dr. Toth's laboratory provide advice and control images for Dr. Guenther's students building OCT systems for brain imaging.

Michael R. Hee Ph.D Massachusetts Institute of Technology

Dr. Hee collaborated on the optical coherence tomographic imaging of retinal laser lesions.

Joseph Izatt, PhD Case Western Reserve University

Dr. Toth is collaborating with Dr. Izatt to improve OCT imaging of the retina. Dr. Toth is a co-investigator with Dr. Izatt (the p.i.) on a "Partnerships in Bioengineering Grant" (NIH 5R24EY-13015-02) In this project, they are

developing a high resolution OCT system that can be used to image retina during surgery or laser delivery.

J. Michael Jumper, MD Wilford Hall, Lackland AFB

Dr. Toth acted as a consultant to Dr. Jumper while he was designing a laser study at the animal research facility at Wilford Hall. Dr. Jumper is utilizing the pig eye as a research model to study the effects of surgical lasers on the retina.

PUBLICATIONS:

1. **C.A. Toth**, D.G. Narayan, C.P. Cain, G.D. Noojin, K.P. Winter, B.A. Rockwell, W.P. Roach. "Pathology of macular lesions from subnanosecond pulses of visible laser energy." Investigative Ophthalmology & Visual Science. 38(11):2204-2213, October, 1997.

2. C.P. Cain, **C.A. Toth**, G.D. Noojin, V. Carothers, DJ. Stolarski, and B.A. Rockwell "Thresholds for visible lesions in the primate eye produced by ultrashort near-infrared laser pulses." Investigative Ophthalmology & Visual Science. 40:2343-2349, Sept, 1999.

3. C.P. Cain, **C.A. Toth**, C.D. DiCarlo, C.D. Stein, G.D. Noojin, D.J. Stolarski, and W.P. Roach. "Visible retinal lesions from ultrashort laser pulses in the primate eye." Investigative Ophthalmology & Visual Science. 36(5):879-888, 1995.

4. **C.A. Toth**, K.P. Winter, M.N. McCall, B.A. Rockwell, C.P. Cain. "Histopathology of ultrashort laser retinal damage: changing retinal pathology with variation in spot-size for near-infrared lesions." Laser-Tissue Interaction X, Steven L. Jacques, Ed., SPIE Proceedings. 3601:32-38, 1999.

5. **C.A. Toth**, E. Worniallo, S.F. Bailey, B.A. Rockwell, C.P. Cain. "A method of achieving three-dimensional reconstruction of tissue at the ultrastructural level demonstrating the distribution of melanosomes within retinal pigment epithelium." Laser-Tissue Interaction X, Steven L. Jacques, Ed., SPIE Proceedings. 3601:11-21 1999.

6. D.J. Stolarski, C.P. Cain, **C.A. Toth**, G.D. Noojin, B.A. Rockwell. "Multiple pulse thresholds in live eyes for ultrashort laser pulses in the near-infrared." Laser-Tissue Interaction X, Steven L. Jacques, Ed., SPIE Proceedings. 3601:22-26, 1999.

7. M.N. McCall, C.J. Harkrider, V. Deramo, S.F. Bailey, K.P. Winter, B.A. Rockwell, D.J. Stolarski, **C.A. Toth**, "Using Optical Coherence Tomography to Elucidate the Impact of Fixation on Retinal Laser Pathology." Laser-Tissue Interaction X., Steven L. Jacques, Ed., SPIE Proceedings. 4257:142-148, 2001.

8. C.D. DiCarlo, W.P. Roach, D.A. Gagliano, S.A. Boppart, D.X. Hammer, A.B. Cox, J.G. Fujimoto, "Comparison of Optical Coherence Tomography Imaging of Cataracts with Histopathology". J. of Biomedical Optics 4(4):450-458, 1999.

9. G.D. Noojin, C.P. Cain, **C.A. Toth**, D.J. Stolarski, M.D., B.A. Rockwell. "Comparison of retinal damage thresholds of laser pulses in the macula/paramacula regions of the live eye." Laser-Tissue Interaction X, Steven L. Jacques, Ed., SPIE Proceedings. 3601:39-42, 1999.

10. B.A. Rockwell, **C.A. Toth**, W.P. Roach, D.J. Payne, R.A. Hopkins, Jr., P.K. Kennedy, D.J. Stolarski, G.D. Noojin, R.J. Thomas, C.P. Cain. "Retinal damage mechanisms and safety for ultrashort laser exposure." Laser-Tissue Interaction X, Steven L. Jacques, Ed., SPIE Proceedings. 3601:4-10,1999.

11. C.P. Cain, **C.A. Toth**, R.J. Thomas, G.D. Noojin, V. Carother, D.J. Stolarski, B.A. Rockwell, "Comparison of Macular Versus Paramacular Retinal Sensitivity to Femtosecond Laser Pulses". J. Biomedical Optics 5(03): 315-320, 2000.

12. R.J. Thomas, G.D. Noojin, D.J. Stolarski, G.T. Hengst, **C.A. Toth**, W.P. Roach, B.A. Rockwell, "Retinal Damage from Femtosecond to Nanosecond Laser Exposure". Laser-Tissue Interaction XI, Steven L. Jacques, Ed., SPIE Proceedings. 3902:54-61, 2000.

13. B.A. Rockwell, **C.A. Toth**, D Stolarski, G Noojin, P Kennedy, J. Shaver, G.D. Buffington, R. Thomas, "Retinal Damage Thresholds for 40 Femtosecond Laser Pulses". Laser-Tissue Interaction XII, Steven L. Jacques, Ed., SPIE Proceedings. 4257:117-124, 2001.

14. **C.A. Toth**, E. Worniallo, S.Bailey, B.A. Rockwell, C.P. Cain, "Methods of Achieving Three Dimensional Reconstruction of Tissue at the Ultrastructural Level Demonstrating the Distribution of Melanosomes within Retinal Pigment Epithelium". Laser-Tissue Interaction X, Steven L. Jacques, Ed., SPIE Proceedings. 3601:11-21, 1999.

Manuscripts in Progress:

Attachment 1. R.J. Thomas, G.D. Noojin, D.J. Stolarski, R.T. Hall, C.P. Cain, **C.A. Toth**, B.A. Rockwell, "A Comparative Study of Retinal Effects from Continuous Wave and Femtosecond Mode-Locked Lasers." Lasers in Surgery and Medicine, *In Submission*.

Attachment 2. C.P. Cain, **C.A. Toth**, G.D. Noojin, D.J. Stolarski, S. Cora, B.A. Rockwell, "Visible Lesion Threshold Dependence on Retinal Spot Size for Femtosecond Laser Pulses in the Primate Eye." Graefes, *In Review.*

Attachment 3. C.P. Cain, **C.A. Toth**, G.D. Noojin, D.J. Stolarski, R.J. Thomas, B.A. Rockwell. "Visible Lesion Thresholds from Multiple Near-Infrared Ultrashort Laser Pulses in the Retina." *In Submission.*

Attachment 4. W.P. Roach, **C.A. Toth**, D.G. Narayan, K.P. Winter, G.D. Noojin, C.D. DiCarlo, S.A. Boppart, M.R. Hee, R. Birngruber, J.G. Fujimoto, C.P. Cain. "The Retinal

Response to Picosecond Laser Pulses of Varying Energy and Spot Size." IOVS, *In Revision*.

Printed Abstracts:

1. **C.A. Toth**, M.N. McCall, K.P. Winter, D.J. Stolarski, C.P. Cain, G.D. Noojin, R. Thomas, B.A. Rockwell. "Histopathology of Sub-50 Femtosecond Near-Infrared Pulsed Laser Retinal Lesions." IOVS. 42(4):S696, 2001.
2. S. Fekrat, C.L. Haupert, N. Sharara, A. Syed, M. Davidson, H.E. Grossniklaus. "Evaluating the Effect of Laser Power on the Success Rate of Rupturing Bruch's Membrane and Retinal Veins in the Pig Model". IOVS. 41(4):S10, 2000.

VII. INTERACTIONS / TRANSITIONS:

Part A: Participation/presentations at meetings, conferences, seminars...

- SPIE: Progress in Biomedical Optics "Proceedings of: Laser-Tissue Interaction VIII" January 1999. Presented "Optical coherence tomography of the retinal response to ultrashort laser pulses."

- Association for Research in Vision and Ophthalmology (ARVO) May 9-14, 1999. Abstract title: "Ultrashort Laser Pulses Can Produce Selective Retinal Pigment Epithelial Cell Injury."

- SPIE: Progress in Biomedical Optics "Proceedings of: Laser-Tissue Interaction VIII" Charlotte NC September 2000. C. Harkrider, C. Toth. Optical Coherence Tomography Imaging of Retinal Tissues In-vivo and Ex-vivum.

- Association for Research in Vision and Ophthalmology (ARVO) April 28-May 4, 2001. Abstract title: "Histopathology of Sub-50 Femtosecond Infrared Pulsed Laser Lesions."

Part B: Consultative and advisory functions to other laboratories and agencies, especially AF and other DoD laboratories.

March 1999. Prepared proposal to assist in development of Specific Aim I: The new proposal under development was to determine if a 1540-nm laser operating at pulse durations between 100-µsec and 500-fs can induce significant damage to corneal tissue.

Dr. Toth provided a written proposal to Dr. Jeremiah Brown AMRDC, Brooks AFB and to Ben Rockwell AFOSR, Brooks AFB. This proposal outlined the study design and methods to collaborate and to evaluate the clinical efficacy of ultrashort laser treatments on retinal drusen lesions associated with macular degeneration in non-human primates. The further development of this project will depend on the Air Force and Army group decisions regarding proceeding with this study at Brooks.

Specific Aim III and IV: We presented a summary of data regarding porcine model use benefits and drawbacks for evaluation of ultrashort laser to retinal-tissue interaction.A notebook with references and the summary report was provided to B. Rockwell (AFOSR) and B. Stuck (AMRDC) (10/30/98)

April 10-11, 2000: Air Force Grant Work, San Antonio, Texas: Dr. Toth presented a histopathology update from the long-term eyes, multiple pulse eyes, and discussed collecting further data points. Dr. Toth also discussed chronic light damage and the drusen study.

Chauhan and Marshall, in 1999, proposed that fixed retinal tissue imaged similar to living tissue on optical coherence tomography. This was an important claim, since OCT could be used to identify laser lesions in sections of fixed tissue. We tested this claim with evaluation of porcine eyes in our laboratory and compared this to imaging

of fixed primate tissue from Armstrong Laboratory in 2000. We found significant impact of fixation on retinal imaging by OCT. We also could identify retinal laser lesions using OCT in excised tissue from Armstrong Laboratory. This was reported to B. Rockwell and a presentation and manuscript was prepared for SPIE January 2001. This was subsequently summarized at the January 2001 AFOSR Ultrashort Laser Group Meeting.

Part C: Transitions:

1998-2001 Basic Research TechnologyTransitions: F46920-95-1-0226 Cynthia A. Toth, M.D., Duke University

Sub Area	Title	PM	Performer	Customer	Result	Application
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr Benjamin A. Rockwell AL/OEDL, Brooks AFB, TX (210)536-4790	Transmission electron micrographs of ultrashort effects on the RPE demonstrate striate and fractured melanosomes	Data supported the concept of microbubble effects as well as LIB.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr. Dave Sliney, (410)671-3932, Am. Conf. of Govt & Industrial Hygienists (ACGIH)	Comprehensive histopathology documenting the minimal level of pathologic response to ultrashort laser pulses in IOVS publication	Comprehensive data to be used in establishing national laser safety standards, i.e., the maximum permissible exposure (MPE) limits of ultrashort laser pulses
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr Benjamin Rockwell, AL/OEDL, Brooks AFB, TX (210)536-4790	Histopathology data demonstrating choroidal damage from the first infrared laser retinal injuries	Data demonstrate a different retinal effect at short pulsewidths with wavelength dependence
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr. Charles Lin Wellman Labs 617-724-3957	Light micrographs of ultrashort primate lesions show lifting and vacuolization which supports his microbubble theory	Photomicrographs provided information to assisted in understanding the clinical effects of microbubble theory
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr. Charles Lin Wellman Labs 617-724-3957	Melanosome response to femtosecond laser pulses includes ruptured and striated melanosomes in moderate lesions	Data assisted in his evaluation of aqueous versus melanosome based plasma formation versus bubble formation
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr. Clarence P. Cain The Analytic Science Corp San Antonio, TX (210) 536-4794	Fluorescein angiography data on laser lesions of primate retina	Data changed the previously held concept that fluorescein angiograms are more sensitive for the identification of retinal injury and support novel theories of laser effects
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Bobbie Geunther, PhD Duke FEL facility 919-660-2674	Overview of pulsed laser effects on ocular tissue	Demonstrated potential benefits of OCT and histopathology in FEL studies
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr. Steve Jacques UT Health Science Center	Melanosome response to femtosecond laser pulses: striated melanosomes are also present in moderate lesions	Melanosome data utilized for theoretical calculations of stress confinement effects from femtosecond laser pulses
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr. Randy Glickman UT Health Science Center	Melanosome response to femtosecond laser pulses: striated melanosomes are also present in moderate lesions	Melanosome data may further modify the theory of superoxide injury from melanosome rupture at threshold
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	International Ophthalmic community	Clinicopathologic correlation of laser retinal lesions and optical coherence tomography	Demonstrated a novel method of analysis of ocular tissue response to laser injury in a publication
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr Benjamin Rockwell, AL/OEDL, Brooks AFB, TX (210)536-4790	Optical Coherence Tomography imaging of the retina is affected by tissue fixation.	Demonstrated that fixation artifact, although not visible with histopathology , is visible on OCT.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr Benjamin A. Rockwell AL/OEDL, Brooks AFB, TX (210)536-4790	Porcine tissue is safer, more readily available, and more cost effective to use as a human model than primate tissue.	Demonstrated that there is an alternative animal model for humans.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr Benjamin A. Rockwell AL/OEDL, Brooks AFB, TX	Histopathology data demonstrating the long-term pathological effects of near-infrared laser pulses.	Data showed the healing effects after laser damage to the retina.

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X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	International Ophthalmic & Engineering community	Adapter arm for the commercial OCT model that allows bench-top imaging of lased tissue.	Demonstrated a new model of analysis for fixed tissue.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	William P. Roach AFOSR	Milestones in SPIE	Assisted Dr. Roach with gathering of background and papers regarding ultrashort laser pulses for Milestones book.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	International Ophthalmic community	Histopathologic analysis of normal, enzyme-treated and lased vitreous.	Demonstrated a need for new techniques to image the vitreous.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr Benjamin Rockwell, AL/OEDL, Brooks AFB, TX (210)536-4790	Histopathology data demonstrating the effects of acute and chronic mode-locked and continuous wave lesions.	Data demonstrates the different retinal effects of mode-locked versus continuous wave laser.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr Benjamin Rockwell, AL/OEDL, Brooks AFB, TX (210)536-4790	Histopathologic data demonstrating the effects of sub-50 femtosecond laser pulses.	Data demonstrated that sub-50 chirped and flat-phase lesions show more localized, smaller areas of damage with no choroidal effect.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Mike Jumper, MD Wilford Hall 59MDW/MMKT Lackland AFB, TX (210) 292-6569	Histopathology study of sub-50 femtosecond laser effects	Assisted in placement of sub-50 fs laser lesions .
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Dr. Joseph Izatt Case Western Reserve University Cleveland, OH (216) 844-7928	High resolution Optical Coherence Tomography	Developed a high resolution OCT that can be used to image retina during surgery or laser delivery.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Thomas Eurell, DVM, Ph.D, Diplomate A.B.T College of Veterinary Medicine, University of Illinois (217)333-7905	Mechanisms of Laser-Ocular Tissue Interaction	Consulted/provided background information for proposed research of cellular and molecular mechanisms governing the ocular tissue response to ultrashort (ps & fs) pulse laser exposure.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Sharon Fekrat, M.D. Duke University Eye Center (919) 681-0341	Laser methods to treat vein occlusions.	Provided information on using a porcine model to test novel new laser methods to create retino-choroidal anastamatosis after vein occlusion.
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Maj. Jeremiah Brown, MS, M.D. Walter Reed Army Institute of Research 7914 A Drive, Bldg. 176 Brooks AFB, TX (210) 536-4622	Laser treatment of drusen lesions associated with macular degeneration in non-human primates.	Provided a written proposal outlining the study design and methods to evaluate the clinical efficacy of ultrashort laser treatments on retinal drusen lesions associated with macular degeneration in not-human primates .
X	Ultrashort laser bioeffects	Kozumbo	Cynthia A. Toth, M.D. Duke University Eye Center (919) 684-5631	Maj. Cheryl D. DiCarlo, USUHS, Department of Anatomy & Cell Biology Bethesda, MD (301) 295-1559	Optical Coherence Tomography Imaging.	Knowledge gained from OCT anterior chamber work (8) was brought to the MFEL for use in our fibrin anterior segment study.

VIII. NEW DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES:

Our published comprehensive studies provide much basic data, for the ANSI "Standard for the safe use of lasers" revision to include ultrashort laser pulses. This data will thus also be used for military laser standards. This data will similarly be used by the European community when the "IEC International Standard for the Safety of Laser Products" is revised to include ultrashort laser pulses.

Daniel X. Hammer, Cynthia A. Toth, William P. Roach, Gary Noojin: Laser Surgical Probe for Use in Intraocular Surgery. U.S. Patent issued, #5,738,676, April, 1998.

OCT imaging can be performed on excised retinal tissue to identify the location of laser lesions.

OCT imaging of excised retinal tissue is affected by formalin and glutaraldehyde fixation.

IX. HONORS / AWARDS:

Dr. Toth named to "Best Doctors in America" 1998,1999, & 2000.

Dr. Toth received a secondary appointment as Associate Professor in Biomedical Engineering in the Pratt School of Engineering, Duke University.

Dr. Toth received the Vitreous Society Honor Award 1999.

Dr. Toth was named the Bodenheim Memorial Lecturer, at the Wilmer Eye Institute, Johns Hopkins University, 2001.

Patents Awarded:

William F. Walker, Cynthia A. Toth, Richard E. Davidsen: Kinetic Acoustic Ocular Examination Apparatus and Method, U.S. Patent # 6,039,691, March 21, 2000.

Cynthia A. Toth, Ronald Overaker, Brian Dodge: Intensity Controllable Hand-held surgical light, U.S. Patent # 6,270,491, August 7, 2001.